Int. J. Nucl. Med. Biol. Vol. 12, No. 5, pp. 381–386, 1985
Printed in Great Britain.

0047-0740/85 $3.00 + 0.00

Evaluation of PLED as a Chelating Ligand for the Preparation of Gallium and Indium Radiopharmaceuticals

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(*Received* 20 *June* 1985)

The ^{68}Ga and ^{111}In complexes of PLED (N,N′-dipyridoxylethylenediamine-N,N′-diacetic acid) were prepared and their biodistribution determined as a function of time following i.v. injection into rats. The ^{68}Ga and ^{111}In complexes behaved identically and were rapidly cleared from the blood via the kidneys into the urine. Similar rapid urinary excretion was observed in the gamma images obtained from a stump-tailed macaque injected with ^{111}In-PLED. Paper electrophoresis at pH 7.35 showed a single radioactive peak for ^{111}In-PLED which migrated towards the anode. PLED administered by i.p. injection was found to speed the blood pool clearance of previously administered ^{67}Ga-citrate.

Introduction

The synthesis and metal ion affinities of the new sexadentate ligand N,N′-dipyridoxylethylenediamine-N,N′-diacetic acid (PLED) were recently reported.[1] Metal ion coordination by PLED occurs through two amino nitrogens, two carboxylate groups, and two phenolate groups of nitrogen heterocycles. The latter impart a high specificity for trivalent metal ions such as those of Ga(III), In(III) and Fe(III), as evidenced by the very high stability constants for PLED complexes of these metals (log K values of 36.35, 36.89 and 36.91, respectively).[1] The PLED ligand has a higher overall basicity than related sexadentate phenolate ligands (e.g. HBED, N,N′-bis(2-hydroxybenzyl)ethylenediamine-N,N′-diacetic acid). As a consequence, there exists unusually high concentrations of Ga(III) or In(III)–PLED complex with no net charge in aqueous solution near pH 7.[1] This paper describes the preparation of the ^{68}Ga ($t_{1/2}$ = 68 min)[2] and ^{111}In ($t_{1/2}$ = 2.8 days)[2] complexes of PLED and the evaluation of their potential as radiopharmaceuticals. The acute toxicity of PLED has been estimated (i.p. LD_{50} in mice: 1120 mg/kg as PLED·3HCl·2CH_3OH·3H_2O) in conjunction with studies which showed i.p. PLED to be 0.8 times as effective as i.p. deferioxamine for iron clearance from mice with iron overload.[3]

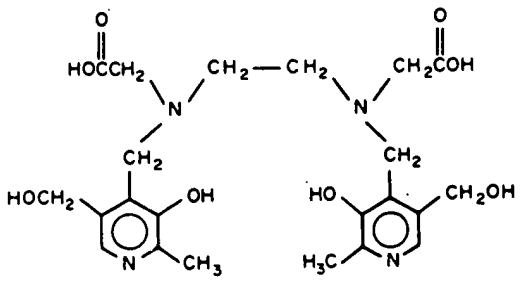
Experimental

General

The PLED ligand was prepared as described previously.[1] For preparation of radiolabelled indium and gallium PLED complexes, aliquots of ligand were taken from a 1.2×10^{-3} M stock solution of PLED in deionized water. No-carrier-added ^{111}In was purchased in 0.9% NaCl solution, pH 1–3, from MediPhysics, Inc. ^{68}Ga was obtained in 1 N HCl solution from a ^{68}Ge/^{68}Ga generator[4,5] available from DuPont/New England Nuclear Corporation. Ethylenediaminetetracetic acid (EDTA) and N-(2-hydroxyethyl)ethylenediaminetriacetic acid (HEDTA) were purchased from Aldrich Chemical Company. Octanol/water partition coefficients were measured as described previously.[6] The distribution of radioactivity on 2.5 cm wide Whatman 1 paper strips following chromatography or electrophoresis was determined using a radiochromatogram scanner interfaced to a strip chart recorder.

Synthesis and characterization of radiolabeled complexes

^{111}In-PLED. In a typical preparation 0.7 mL PLED stock solution and 0.5 mL 1.0 M acetate buffer



PLED

Scheme 1.

(pH 6.8) were added to 1.5 mL acidic saline containing 1 mCi ^{111}In, giving a solution of pH 4.5. The solution was then raised to pH 7 by addition of 0.5 mL 1 M HEPES buffer (pH 7.5). The ^{111}In-PLED solution was filtered through a 0.22 μm Millipore Millex-GS sterile filter before use. The EDTA and HEDTA complexes of ^{111}In were prepared in a similar manner by substitution of the appropriate ligand for PLED.

^{68}Ga-PLED. The generator eluent of 15 mCi ^{68}Ga in 2.5 mL 1 N HCl was evaporated to dryness in a test tube by heating under a stream of dry N_2. The residual ^{68}Ga was redissolved in saline pH 1–3 and treated as described above for the preparation of ^{111}In-PLED.

The purity of the ^{111}In complexes was evaluated by chromatography on Whatman 1 paper eluted[7] with 700 mL H_2O:200 mL ethanol:0.4 mL NH_4OH. The ^{111}In-PLED, ^{111}In-EDTA and ^{111}In-HEDTA complexes all chromatographed as single peaks with R_f values of 0.85 ± 0.05, whereas control experiments showed unchelated ^{111}In(III) remained at the origin ($R_f = 0$). The ^{111}In-PLED, ^{111}In-EDTA and ^{111}In-HEDTA complexes all migrated towards the anode upon electrophoresis using Whatman 1 paper and 1 M HEPES buffer (pH 7.35) electrolyte (samples were run simultaneously at a constant current of 4 mA, *ca.* 100 V, for 90 min).

Animal studies

The biodistributions of ^{111}In-PLED and ^{68}Ga-PLED were determined following a 0.2 mL injection into the femoral vein of ether-anesthetized Sprague–Dawley rats which were sacrificed by decapitation at appropriate time intervals. All animals had free access to food and water prior to sacrifice. Total blood volume was assumed to be 7% of the body weight. Bladder and urine uptake of tracer was determined by removal of the intact full bladder from rats which had undergone penile ligation immediately prior to tracer injection.[8,9] The urine from a rat with penile ligation was analyzed by paper chromatography 60 min following injection of 50 μCi ^{111}In-PLED using two different solvent systems: 700 mL

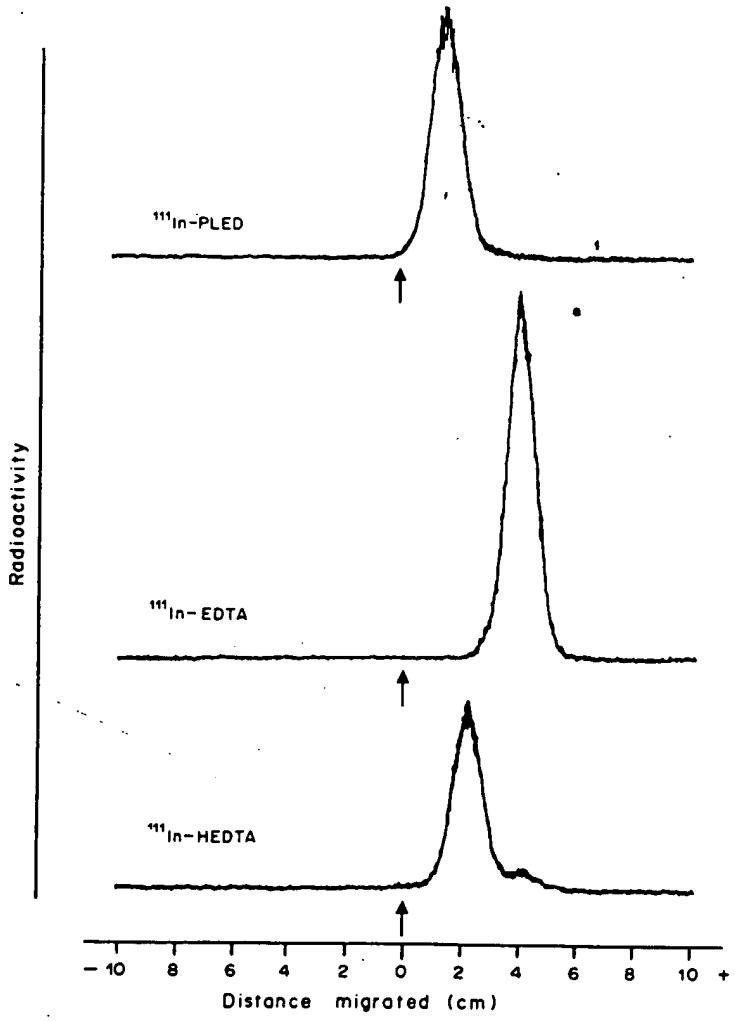


Fig. 1. Radiochromatographs following paper electrophoresis under the conditions described in the text.

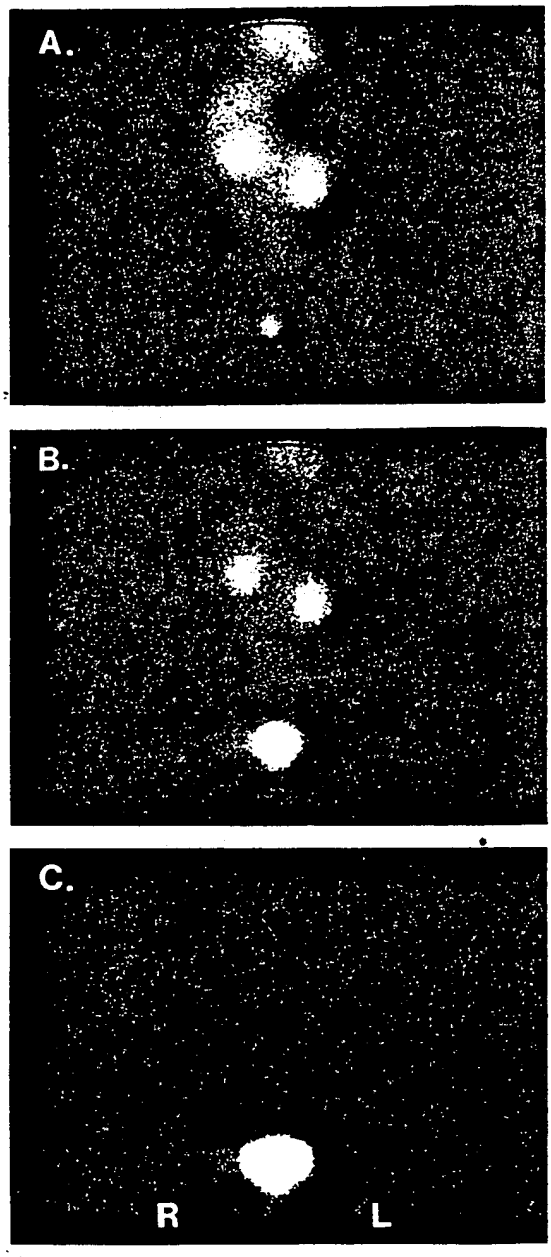


Fig. 2. Gamma images obtained following i.v. injection of ^{111}In-PLED into a stump-tailed macaque showing the kidneys and bladder. (A) Immediately post-injection (0–220 s). (B) Five minutes post-injection. (C) Forty-five min post-injection. The chest of the animal is located at the top of each image.

Table 1. ^{68}Ga-PLED biodistribution in rats

Organ	% Injected dose per organ			
	1 min*	5 min*	15 min**	60 min**
Blood	28.2 ± 2.8	11.4 ± 0.8	9.2 ± 0.8	2.5 ± 0.2
Brain	0.06 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.01 ± 0.002
Heart	0.47 ± 0.04	0.23 ± 0.01	0.18 ± 0.02	0.04 ± 0.01
Lung	1.31 ± 0.18	1.1 ± 0.3	0.60 ± 0.19	0.15 ± 0.05
Liver	2.26 ± 0.82	1.65 ± 0.13	1.30 ± 0.13	0.48 ± 0.04
Spleen	0.22 ± 0.02	0.13 ± 0.02	0.10 ± 0.02	0.03 ± 0.01
Kidney (each)	5.8 ± 1.0	2.7 ± 0.6	2.9 ± 0.5	0.72 ± 0.06
Bladder and urine	—	—	22.8 ± 3.5***	47.0 ± 5.5***

The values shown represent the mean of: * three, ** four or *** five 250–425 g rats.

H_2O:200 mL ethanol:0.4 mL NH_4OH[7] and 70 mL methyl ethyl ketone:30 mL acetic acid.[10] Gamma images from a 13 kg stump-tailed macaque receiving i.m. ketamine and i.v. saline were collected at 1 min intervals for 60 min following i.v. injection of 850 μCi ^{111}In-PLED.

To determine whether the PLED ligand could be used to speed blood pool clearance of ^{67}Ga administered for tumor and abscess imaging, 15–20 μCi ^{67}Ga-citrate (Mallinckrodt, Inc.) was injected into the left femoral vein of each of 17 rats (206–245 g). Four hours following ^{67}Ga injection, 7.5×10^{-6} mol PLED dissolved in 0.2 mL deionized water was injected into the right femoral vein of five of the rats, while two rats received 1.5×10^{-4} mol PLED intraperitoneally. All rats were sacrificed 24 h following ^{67}Ga-citrate injection and the affect of PLED on the gallium distribution determined.

Results and Discussion

Despite the fact that there exists at physiological pH appreciable concentrations of gallium and indium PLED complexes with no net charge, ^{68}Ga-PLED and ^{111}In-PLED were not found to be lipophilic (octanol/water partition coefficients in the range of 10^{-4}–10^{-5}). The hydrophilic nature of these complexes can be explained by the equilibrium concentrations of charged PLED complexes,[1] the possibly zwitterionic nature of the "uncharged" complexes, and the presence of hydrophilic substituents ($-CH_2OH$ and pyridine N) on the ligand backbone. Upon electrophoresis at pH 7.35 ^{111}In-PLED migrated towards the anode (Fig. 1), as would be expected for a complex existing in solution as an equilibrium mixture of neutral and anionic forms.[1]

The ^{68}Ga and ^{111}In complexes of PLED behave as charged species upon i.v. injection into rats. The gallium and indium PLED complexes behaved identically and were rapidly taken up by the kidneys and excreted into the urine. The biodistribution data for ^{68}Ga-PLED and ^{111}In-PLED are given in Tables 1 and 2. The complexes are cleared from the blood indicating, as expected, that exchange to form ^{68}Ga or ^{111}In-transferrin does not occur to a significant extent. At 1 h post-injection, approximately half of the injected dose was found in the bladder and urine of mature rats. Clearance of ^{111}In-PLED was more rapid in immature rats (Table 3), with 86% of the injected dose found in the bladder and urine at 1 h post-injection. The bladder and urine accumulation of ^{68}Ga-PLED and ^{111}In-PLED is somewhat more rapid than that reported for ^{67}Ga-EDTA[11] and is much more rapid than the clearance of the ^{67}Ga complexes of the tricatecholamides 3,4-DiP-LICAM, 3,4-DiP-LICAMS and TiP-MECAMS.[11] The ^{68}Ga and ^{111}In complexes of PLED do not cross the intact blood-brain-barrier or exhibit specificity for any organs other than the kidney.

Paper chromatography of the urine from a rat injected with ^{111}In-PLED suggests that the complex is excreted intact. Using the 700 mL H_2O:200 mL ethanol:0.4 mL NH_4OH solvent system,[7] ^{111}In-PLED and the radioactivity in the urine chromatographed

Table 3. Bladder and urine accumulation of ^{111}In-PLED in immature rats with penile ligation

Time	% Injected dose
15 min*	48 ± 3
60 min**	86 ± 3

* Mean of five rats (97–103 g).
** Mean of eight rats (84–125 g).

Table 2. ^{111}In-PLED biodistribution in rats

Organ	% Injected dose per organ				
	1 min*	5 min	15 min	1 h	3 h
Blood	25.6 ± 1.5	14.0 ± 1.1	7.4 ± 1.2	2.1 ± 1.1	0.07 ± 0.04
Brain	0.055 ± 0.009	0.040 ± 0.009	0.023 ± 0.007	0.010 ± 0.004	0.0017 ± 0.0002
Lung	1.41 ± 0.15	0.87 ± 0.05	0.49 ± 0.04	0.13 ± 0.04	0.017 ± 0.004
Heart	0.43 ± 0.07	0.26 ± 0.05	0.17 ± 0.01	0.04 ± 0.01	0.0025 ± 0.0014
Liver	3.1 ± 0.60	1.6 ± 0.1	0.97 ± 0.10	0.37 ± 0.08	0.13 ± 0.03
Spleen	0.27 ± 0.05	1.133 ± 0.007	0.087 ± 0.007	0.027 ± 0.011	0.009 ± 0.003
Kidney (each)	4.9 ± 1.0	3.4 ± 0.7	2.2 ± 0.2	0.81 ± 0.19	0.27 ± 0.06
Bladder and urine	—	—	23.0 ± 4.0*	52.0 ± 6.0*	—

Values shown represent the mean of four 253–356 g rats.
* Five rats.

Table 4. Effect of PLED on the biodistribution of ^{67}Ga citrate in rats (206–245 g)*

Organ	% Injected dose per gram		
	Control (no PLED)**	PLED (i.v.)***	PLED (i.p.)****
Blood	0.31 ± 0.09	0.25 ± 0.10	0.05 ± 0.01
Bone	2.35 ± 0.63	1.63 ± 0.39	0.82 ± 0.25
Liver	0.81 ± 0.24	0.83 ± 0.12	0.49 ± 0.08
Spleen	0.91 ± 0.31	1.27 ± 0.37	0.47 ± 0.08
Lung	0.29 ± 0.09	0.26 ± 0.10	0.07 ± 0.01
Kidney	1.03 ± 0.32	1.53 ± 0.41	0.79 ± 0.08

* All rats sacrificed 24 h following i.v. injection of ^{67}Ga-citrate. PLED was administered 4 h following ^{67}Ga-citrate by either i.v. or i.p. injection of the specified dose.
** Values shown represent the mean of 10 rats.
*** Values shown represent the mean of five rats. PLED dose: 7.5×10^{-6} mol.
**** Values shown represent the mean of two rats. PLED dose: 1.5×10^{-4} mol.

as single radioactive peaks with $R_f = 0.85 \pm 0.05$ (unchelated ^{111}In would have remained at the origin). Using a second solvent system[10] (70 mL methyl ethyl ketone:30 mL acetic acid) ^{111}In-PLED and the radioactivity in the urine remained at the origin ($R_f = 0$), while in control experiments, unchelated ^{111}In was shown to migrate with $R_f = 0.87$.

The gamma images obtained following i.v. injection of ^{111}In-PLED into a monkey are shown in Fig. 2. The biodistribution of the complex is that which would be expected based on the studies in rats. The kidneys are readily visible and the bladder accumulation with time is apparent. Computer analysis of the data shows that >60% of the activity in the field of view is present in the bladder at 60 min post-injection.

Deferoxamine mesylate[12–16] and the tricatecholamide LICAM-C[17] have been shown in animal studies to be useful for enhancement of ^{67}Ga tumor-to-background and ^{67}Ga-abscess-to-background ratios by displacement of ^{67}Ga from plasma binding sites. To determine if PLED could be used for this purpose, PLED was administered to rats 4 h after ^{67}Ga-citrate injection either intravenously (7.5×10^{-6} mol) or intraperitoneally (1.5×10^{-4} mol). The rats were sacrificed 24 h following ^{67}Ga-citrate injection and the amount of ^{67}Ga in the blood, liver, spleen, lung, kidney, and bone compared with the levels found in control animals which were not given PLED. The results are shown in Table 4. Intravenous PLED did not significantly alter the clearance of ^{67}Ga at this dose (7.5×10^{-6} mol was chosen because that was the amount of LICAM-C used in a previously reported study).[17] Due to limitations in the availability of PLED, only two rats could be studied at the higher i.p. doses shown previously to be effective for iron clearance. A dramatic reduction in the ^{67}Ga-levels of the blood, lung, and bone was apparent following such treatment, with smaller reductions of the ^{67}Ga-levels in the liver and spleen. Sclerosis of the liver was apparent upon sacrifice of the rats receiving this i.p. dose which was only 2–3 times lower than the estimated LD_{50}. The contrast between the effectiveness of i.v. PLED and LICAM-C may reflect the relative rates at which the free ligands are cleared from the blood pool along with the relative rates of metal exchange between transferrin and these multidentate ligands.

Acknowledgements—This work was supported by the United States Department of Energy (DOE) Grant DE-ACO2-77EV04318 (to MJW, MAG and CJM) and the National Cancer Institute, National Institutes of Health, United States, Public Health Service Grant CA22464 (to AEM and RJM).

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